HYBRIDIZATION BETWEEN *ESCHERICHIA COLI* AND SHIGELLA[1]

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Genetic recombination by mating has been demonstrated among a variety of strains classified as *Escherichia coli* (Lederberg and Tatum, 1946; Cavalli *et al.*, 1953). The possibility of genetic transfers among bacteria currently classified in different species or genera has been proved by transformation and transduction experiments (Lederberg and Edwards, 1953; Schaeffer and Ritz, 1955; Lennox, 1955). These findings are hardly surprising since there is no reason to expect that current bacterial classification correlates closely with capacity for hybridization or even with evolutionary relations (Luria, 1947). Hybridization capacity, even if present, might indeed not be responsible for significant amounts of gene flow among natural bacterial populations, which can propagate indefinitely by vegetative reproduction alone. Hence, adaptive selection might lead to considerable diversity among potentially interfertile clones, both because of the rarity of fertilization events and because of other isolation mechanisms.

In this paper we present evidence that places the majority of dysentery bacilli (genus *Shigella*) into the same fertility system as *Escherichia coli*. The results also indicate a possible role of genetic recombination in the origin of some Shigella serotypes and, more generally, in the evolution of natural populations of these bacteria. They suggest some potential pitfalls of current bacteriological procedures for the identification of pathogenic enteric bacteria.

MATERIALS AND METHODS

Cultures. E. coli strain K-12 and its derivatives were from our laboratory collection. Most of these strains were received from Drs. L. Cavalli, W. Hayes, E. M. and J. Lederberg, P. D. Skaar, and E. Wollman. *E. coli* strain C (Lieb *et al.*, 1955) was also used. All the strains are motile.

Shigella dysenteriae strain Sh is a rough strain, commonly used as indicator for the phages carried by *E. coli* strain Lisbonne-Carrère (Bertani, 1951). Type cultures of *S. dysenteriae, S. flexneri,* and *S. boydii* were received either from the Division of Laboratories, Illinois Department of Public Health, Chicago, thanks to Dr. H. J. Shaughnessy, or from the Communicable Disease Center, U. S. Public Health Service, Atlanta, through the courtesy of Dr. W. H. Ewing. The Illinois strains are identified by the symbol I, the Atlanta strains by the symbol A (for example, *S. dysenteriae* strain 1I; *S. flexneri* strain 2aA).

Streptomycin resistant mutants (S^r) were isolated from plates of solid nutrient media containing 100 μg/ml streptomycin that had been seeded with about 10^{10} streptomycin sensitive (S^s) cells per plate. S^r cultures were maintained and grown with 100 μg of streptomycin/ml, except when this would interfere with the experimental procedure. Phage resistant mutants were isolated by standard methods. Lysogenic derivatives were obtained from plates with confluent incomplete lysis. Phage sensitivity tests were done by cross smearing.

Media. L broth and L agar contain: tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; glucose, 1 g; water, 1,000 ml; (pH 7.0) with or without 10 g powdered agar, respectively. Minimal, EMB and EMS (= minimal EMB) media were prepared following Lederberg (1950a) with 15 g/L agar. Sugars were dissolved in water, sterilized and added to media to a concentration of 1 per cent. Media for gas production tests were made by adding 1 per cent sugar (sterilized

separately) to tubes of nutrient broth (pH 7.2) with bromthymol blue indicator and inverted vials. Assays of phage λ were done on the medium recommended by Kaiser (1955). Phage P1 was assayed on L agar plus 10^{-2} M $CaCl_2$.

Antisera. Sera against Shigella type cultures were obtained either commercially (Markham Laboratories, Inc.) or through the courtesy of Dr. W. H. Ewing and Dr. I. Saphra. Sera (anti-O) against *E. coli* strain K-12, its derivatives, and hybrid strains were prepared in rabbits by 1 or 2 injections of about 10^9 cells collected from nutrient agar, heated at 100 C for 2.5 hr and mixed with adjuvants (Freund's method, see Cohn, 1952). Adsorbed sera were prepared by the double adsorption method according to Edwards and Ewing (1955) using cells from 10 plates of L agar/ml of serum.

Agglutination tests were done in 9 mm tubes, using 0.5 ml vol of saline. The tubes were kept at 56 C for 2 hr, then at room temperature overnight. The antigens consisted of cells collected from agar slants or from heavy broth cultures, with or without washing. Whether used alive, or heated for 1 hr at 100 C, or treated with 75 per cent ethyl alcohol or with 0.5 per cent formalin overnight, the cells of a given organism gave O-agglutination endpoints agreeing within a factor of 2. In most tests, formolized antigens were used for nonmotile cultures and alcohol treated antigens for motile ones. A rapid diagnostic test for serological changes in hybrids will be described in a later section.

RESULTS

Selection of recombinants. Strains to be crossed must exhibit stable differences in characters so that recombinants can be enriched and detected selectively. A convenient method proved to be selection for lactose utilization in streptomycin containing media (Lederberg, 1951; Hayes, 1953b). Recombinants Lac⁺Sʳ can be obtained from mixtures of Sʳ Shigellas and Sˢ (streptomycin sensitive) *E. coli* parents plated on EMB lactose agar with 100 to 200 μg/ml streptomycin. All Shigella strains proved stably Lac⁻; plated alone, they gave no Lac⁺ papillae on EMB-lactose agar and no detectable growth in minimal media with lactose as only carbon source (in addition to necessary growth factors); nor was any β-galactosidase detectable in these strains by the o-nitrophenyl-β-D-galactoside test (Leder-

berg, 1950b). Sʳ cells appear with a frequency lower than 10^{-9} in Sˢ cultures of *E. coli* strain K-12 derivatives.

Sʳ derivatives of the Shigella strains to be tested were isolated first. Heavy suspensions from stationary cultures of these Shigellas (Lac⁻Sʳ) and of *E. coli* (Lac⁺Sˢ), grown in L broth to about 10^9 cells/ml, chilled and collected by cold centrifugation, were mixed directly on EMB-lactose streptomycin agar. Lac⁺ papillae, when present, appeared in 2 to 3 days. These papillae, upon isolation by streaking on EMB lactose, yielded stable Lac⁺Sʳ clones, whose recombinant nature was confirmed by tests for other characters in which the parent strains differed (unselected markers, see below). The Lac⁺ recombinants gave a positive test for β-galactosidase. They produced acid from lactose in 18 to 24 hr (pH 4.9 ± 0.2) and no gas.

The parental combinations that were tested by this method are shown in table 1. The parental Shigella strains never yielded a single Lac⁺ mutant in any of the control plates, which contained altogether over 10^{11} cells. Note that Lac⁺ recombinants were obtained also in crosses of Shigella with *E. coli* strain C, a strain which differs from K-12 in many respects (Lieb *et al.*, 1955).

Frequency of recombination and polarity. The frequency of recombinants Lac⁺Sʳ (or Lac⁺ prototrophs, see below) as a function of the polarity (Hayes, 1953a; Cavalli *et al.*, 1953) of the *E. coli* parents is shown in table 2. In these and in all other crosses, the Shigella parents behave as typical F⁻ strains. All Hfr cultures of *E. coli* (see Hayes, 1953b) give higher frequencies of recombination than F⁺ cultures with Shigellas as well as with other strains of *E. coli*. Thus, the mating polarity system observed in *E. coli* extends to Shigellas as well. The frequencies of recombinants are not much higher when the parents are incubated together before plating than when they are mixed on plates. The frequencies are uniformly lower for crosses of F⁺ or Hfr *E. coli* with Shigellas than with F⁻ *E. coli*. It will be shown below that this difference is due to lower effectiveness of matings in producing recombination rather than to lower frequency of mating.

Unselected markers and linkage. Recombinants isolated from crosses are expected to contain various assortments of the genetic traits, other

TABLE 1

Fertile combinations of strains as tested by the Lac⁺Sʳ selection

Samples of 0.1 ml from each culture were mixed directly on EMB-lactose-streptomycin agar. Lac⁺ colonies were isolated by restreaking on the same medium.

Organism	Strain	Organism	Strain	Recombinants
Shigella dysenteriae Sh	× *Escherichia coli*	K-12 F⁺		+
S. dysenteriae	Sh	× *E. coli*	K-12 Hfr	+
S. dysenteriae	1I*	× *E. coli*	K-12 Hfr	+
S. dysenteriae	2A†	× *E. coli*	K-12 Hfr	+
S. dysenteriae	3I	× *E. coli*	K-12 Hfr	+
S. dysenteriae	3A	× *E. coli*	K-12 Hfr	+
S. dysenteriae	4I	× *E. coli*	K-12 Hfr	+
S. dysenteriae	4A	× *E. coli*	K-12 Hfr	+
S. dysenteriae	5I	× *E. coli*	K-12 Hfr	+
S. dysenteriae	8A	× *E. coli*	K-12 Hfr	+
Shigella flexneri	1aI	× *E. coli*	K-12 Hfr	+
S. flexneri	2aI	× *E. coli*	K-12 F⁺	+
S. flexneri	2aI	× *E. coli*	K-12 Hfr	+
S. flexneri	2aA	× *E. coli*	K-12 F⁺	+
S. flexneri	2aA	× *E. coli*	K-12 Hfr	+
S. flexneri	2bA	× *E. coli*	K-12 F⁺	+
S. flexneri	2bA	× *E. coli*	K-12 Hfr	+
S. flexneri	4aI	× *E. coli*	K-12 Hfr	+
S. flexneri	4aA	× *E. coli*	K-12 Hfr	+
Shigella boydii	4A	× *E. coli*	K-12 F⁺	+
S. boydii	4A	× *E. coli*	K-12 Hfr	+
S. boydii	6A	× *E. coli*	K-12 F⁺	+
S. boydii	6A	× *E. coli*	K-12 Hfr	+
Shigella flexneri	2aI	× *E. coli*	C F⁺	+
S. flexneri	2bA	× *E. coli*	C F⁺	+

* I = strains from the Division of Laboratories, Illinois Department of Public Health, Chicago, Illinois.

† A = strains from the Communicable Disease Center, U.S.P.H.S., Atlanta, Georgia.

than the selected ones, in which the parent strains differed ("unselected markers"). Generally, more characters are contributed to the hybrids by the F⁻ parent than by the F⁺ or Hfr parent (Hayes, 1953b). The relative frequencies of various unselected markers and their dependence on the choice of selective markers make it possible to map the corresponding genetic factors in a linear linkage map (Lederberg, 1947).

In the present work we have not attempted to construct a detailed genetic map of any one strain of *Shigella*. A number of characters were used either as selective or as unselected markers, mainly in crosses between *E. coli* strain K-12, *S. dysenteriae* strain Sh and *S. flexneri* strain 2aI and their derivatives. The characters that were tested for presence or absence in hybrids are listed in table 3. As expected, the hybrids are predominantly like the Shigella F⁻ parents. Note that some characters from the F⁺ or Hfr coli parents never appeared in the hybrids. This is true for production of indole, for resistance to azide, and for utilization of xylose, maltose and mannitol with *S. dysenteriae* strain Sh; for motility and gas production both with strain Sh and with *S. flexneri* strain 2aI.

Any character that appears in hybrids as a nonselected marker can also be used as a selective marker. For example, selection was successful for Ara⁺Sʳ hybrids in crosses of *S. dysenteriae* strain Sh × *E. coli*, and for Xyl⁺Sʳ (or Dul⁺Sʳ, or Rha⁺Sʳ) with *S. flexneri* strain 2aI. All Ara⁺ and Xyl⁺ recombinants produce acid in 24 hr

TABLE 2

Frequency of recombination and polarity of the Escherichia coli parent

Examples of recombination frequencies obtained in crossing Shigella cultures with derivatives of *E. coli* strain K-12.

Experiment	*Shigella* and *Escherichia coli* Parents: Organism, Strain	Selection for	Ratio Recombinants/ Hfr or F$^+$ cells
I* 1-31-55	*S. dysenteriae* 57-1 prototroph × *E. coli* F$^-$M$^-$	Lac$^+$M$^+$	<10^{-9} (none)
	S. dysenteriae 57-1 prototroph × *E. coli* F$^+$C$^-$H$^-$	Lac$^+$C$^+$H$^+$	10^{-7}
	S. dysenteriae 57-1 prototroph × *E. coli* Hfr M$^-$S*	Lac$^+$M$^+$	2 × 10^{-5}
	S. dysenteriae 57-1 prototroph × *E. coli* Hfr M$^-$S^r	Lac$^+$M$^+$	4 × 10^{-5}
II† 1-8-57	*S. dysenteriae* Sh S^r × *E. coli* F$^+$(λ) prototroph S*	Lac$^+$S^r	1.4 × 10^{-7}
	S. flexneri 2aI S^r (λ) × *E. coli* F$^+$(λ) prototroph S*	Lac$^+$S^r	6.6 × 10^{-7}
	E. coli F$^-$T$^-$L$^-$S^r (λ) × *E. coli* F$^+$(λ) prototroph S*	T$^+$L$^+$S^r	1 × 10^{-5}
III‡ 12-13-56	*S. dysenteriae* Sh S^r × *E. coli* Hfr S*	Lac$^+$S^r	6 × 10^{-4}
	S. flexneri 2aI S^r × *E. coli* Hfr S*	Lac$^+$S^r	3 × 10^{-4}
	E. coli F$^-$T$^-$L$^-$S^r × *E. coli* Hfr S*	T$^+$L$^+$S^r	7 × 10^{-1}

* Cultures mixed directly on plates of EMS-lactose-agar.

† Mixtures containing 3-12 × 10^8 F$^-$ and 3.0 × 10^8 F$^+$ cells; incubated for 40 min with aeration before plating. Assays after 30 min of aeration.

‡ Mixtures containing 5-8 × 10^9 F$^-$ cells and 7 × 10^8 Hfr cells; incubated for 40 min in broth without aeration before plating. In this experiment the number of Hfr cells was measured at the time of mixing.

Platings in experiments II and III were done on EMB-lactose-streptomycin-agar for the Shigella × *E. coli* crosses; on minimal-thiamine-streptomycin-agar for the *E. coli* × *E. coli* crosses. The medium used in the Shigella × *E. coli* crosses may allow additional recombination on the plates.

Symbols: T = threonine; L = leucine; M = methionine; C = cysteine; H = histidine; S = streptomycin.

TABLE 3

Transmission of characters from Escherichia coli parents to hybrids

Shigella Parent: Organism, Strain	Characters from *E. coli* Parent Tested in Hybrids		
	Selected	Unselected	
		Present	Absent
S. dysenteriae, Sh (or 57-1): Lac$^-$ Ara$^-$ Xyl$^-$ Mal$^-$ Mtl$^-$ S^r V$_{1.5}$* V$_6$* Az* Ind$^-$ P$_1$^{r} λ^r mot$^-$ aer$^-$	Lac$^+$ Lac$_1$$^-$ Ara$^+$	V$_{1.5}$^{r}; V$_6$^{r}; Ara$^+$; S*. V$_{1.5}$^{r}. Lac$^+$; S*.	Mal$^+$; Mtl$^+$; Xyl$^+$; Az^r; Ind$^+$; P1$^+$; λ$^+$; mot$^+$; aer$^+$
S. flexneri, 2aI: Lac$^-$ Xyl$^-$ Rha$^-$ Dul$^-$ V$_{1.5}$* λ·C Nic$^-$ Glt$^-$ mot$^-$ aer$^-$	Lac$^+$ Xyl$^+$ Rha$^+$ Dul$^+$	V$_{1.5}$^{r}. Lac$^+$; V$_{1.5}$^{r}. Lac$^+$.	V$_6$^{r}; (Nic Glt)$^+$; mot$^+$; aer$^+$; λ·K

Code: Lac = lactose. Ara = arabinose. Xyl = xylose. Rha = rhamnose. Dul = dulcitol. Mal = maltose. Mtl = mannitol. Ind = indole production. mot = motility. aer = gas production. Az = azide. S = streptomycin. Nic = nicotinamide. Glt = glutamate. V$_{1.5}$ = phages T1, T5. V$_6$ = phage T6. P1 = Phage P1. λ·C = phage λ produced like that from *E. coli* strain C. λ·K = phage λ produced like that from *E. coli* strain K-12. Lac$_1$$^-$ = cryptic. β-D-galactosidase. $^+$ = utilized; or synthesized; or lysogenic; or present. $^-$ = not utilized; not synthesized; not lysogenic; or absent. * = sensitive. ^{r} = resistant.

(and no gas) in liquid media with the corresponding sugars.

S. flexneri strain 2aI grows in minimal medium supplemented with nicotinamide and glutamate (Nic⁻Glt⁻). In crosses of *S. flexneri* strain 2aI with auxotrophic F⁺ or Hfr strains of *E. coli* (M⁻ or T⁻L⁻ or C⁻H⁻) no prototrophic recombinants were observed. Crosses on minimal agar + nicotinamide + glutamate + lactose yielded only Lac⁺ Nic⁻ Glt⁻ recombinants. Thus, there was no evidence for transfer of Nic⁺ and Glt⁺ from *E. coli* to hybrids.

S. dysenteriae strain Sh has ill-defined, nonspecific requirements for a variety of amino acids, but it readily yields variants that grow fairly well on minimal medium. One such variant, strain 57-1, obtained from a Sʳ derivative of strain Sh, has been used extensively in our work. In crosses of Shigella 57-1 × *E. coli*, we can select hybrids either for Lac⁺Sʳ or for Lac⁺ prototrophy (on EMS minimal agar with lactose as only carbon source) as shown in table 2. The latter selection allows the isolation of recombinants with the Sˢ character from an *E. coli* parent.

The frequency of unselected markers permits us to establish a few linkage relations. Crosses of Shigella Sh (or 57-1) × *E. coli* suggest the following linkage pattern: Ara—V₁, ₛ—Lac—V₆ (or possibly Ara—V₁, ₛ—V₆—Lac). Here Ara and Lac represent the loci whose alleles are present in the Shigella parent. In crosses with *S. flexneri*, Lac is closely linked to V₁, ₛ and only distantly linked to Rha and Xyl. The linkage relation between the loci V₁, ₛ, Lac, V₆ and Ara is similar in Shigella and in *E. coli* strain K-12 (Cavalli-Sforza and Jinks, 1956). This indicates extensive homology of genetic organization.

It is impossible to decide, on the basis of our present data, whether the characters of the F⁺ (or Hfr) coli parents that fail to appear in hybrids are excluded by the choice of selective markers (linkage with markers against which selection was made), or by incomplete homology between the linkage systems of *E. coli* and Shigella, or by chromosomal rearrangements. Some characters, such as motility and aerogenesis, may involve multigene control. Failure of expression of a gene may be due to interaction with other genetic factors. These problems may

be resolved more easily by use of transduction (Lennox, 1955).

An interesting cross, included in table 3, is one in which the *E. coli* parent carries the mutation Lac₁⁻ (cryptic β-galactosidase) (Lederberg *et al.*, 1951). Adaptive synthesis of β-galactosidase can be induced more readily by alkyl-β-galactosides than by lactose. Crosses on EMB-lactose-streptomycin agar yield late papillae, from which one can isolate bacteria that form β-galactosidase when exposed to methyl-β-galactoside. Thus, the hybrid has acquired the same Lac₁⁻ allele present in the *E. coli* parent.

Behavior towards phages P1 and λ. Strains of *E. coli* and of Shigella are hosts for many common phages (including the T1-T7 phages), with individual differences in host range. A special interest applies to phages P1 and λ, the former as an agent of transduction (Lennox, 1955), the second as a phage whose prophage location in the linkage map of *E. coli* strain K-12 and whose behavior in bacterial crosses have been investigated (Wollman *et al.*, 1956).

The host range of these phages among Shigella cultures is shown in table 4. The widespread sensitivity to phage P1 opens the possibility of genetic analysis by means of transduction. Note also that some Shigella strains, like *S. dysenteriae* strain Sh, are susceptible to P1 with its original host range as first isolated from *E. coli* strain Lisbonne-Carrère (Bertani, 1951); other strains, like *S. flexneri* strain 2aI, are only lysed by the mutant P1*k*, isolated by its ability to lyse *E. coli* strain K-12. This observation suggests further mutational homologies between Shigellas and *E. coli*.

Phage λ attacks only *S. flexneri* strain 2aI; the other Shigellas are neither lysed nor lysogenic for λ. *S. flexneri* strain 2aI is readily lysogenized. When λ is liberated by this strain, either by induction of a lysogenic culture or by infection of a sensitive culture, it is modified in the same way as when grown on *E. coli* C (Bertani and Weigle, 1953); this λ·C phage fails to grow in most cells of (nonlysogenic) *E. coli* strain K-12 derivatives. This indicates one additional genetic homology between a Shigella and *E. coli* strain C. Several hybrids from strain 2aI × *E. coli* strain K-12 were tested and all retained the C-like modifying property.

Lysogenic derivatives were used in most

TABLE 4

Sensitivity of various strains to phages P1 and λ

Organism	Phage P1		Phage λ		Kind of Phage λ Produced
	Wild type	λ Mutant, active on K-12	Grown on E. coli K-12	Grown on E. coli C	
Escherichia coli K-12	R	S	S	s	λ·K
E. coli C	S	S	S	S	λ·C
Shigella dysenteriae Sh	S	S	R	R	
S. dysenteriae 1A	R	R			
S. dysenteriae 2A	R	R	R	R	
S. dysenteriae 5A	R	R	R	R	
S. dysenteriae 6A	R	R	R	R	
S. dysenteriae 7A	R	R	R	R	
S. dysenteriae 8A	R	S	R	R	
S. flexneri 2aI and hybrids	R	S	S	S	λ·C
S. flexneri 2aA and hybrids	S	S	R	R	
S. flexneri 2bA	R	S	R	R	
S. flexneri 4aA	S	S	R	R	
S. boydii 4A	R	R	R	R	
S. boydii 6A	S	S	R	R	

S = sensitive; R = resistant by spot test; s = sensitive with low efficiency of plating; A = type strains from Atlanta; I = type strains from Chicago.

crosses of *S. flexneri* strain 2aI with λ lysogenic *E. coli* strain K-12 in order to avoid "zygotic induction" (Jacob and Wollman, 1956), that is, maturation of λ and lysis of the F⁻ parent when prophage λ is transferred from a lysogenic F⁺ or Hfr mate.

The phenomenon of zygotic induction of λ made it possible (Wollman *et al.*, 1956) to estimate the frequency of mating, as distinct from the frequency of recombination, which depends on the frequencies of mating, of transfer of the selective markers from the F⁺ or Hfr parent, and of postzygotic integration of these markers into the genome of the hybrids. Experiments on zygotic induction were done with *E. coli* culture Hfr H B₁⁻ (λ), a strain that was used extensively by Wollman *et al.* (1956) in their analysis of the mechanism of recombination, details of which need not be given here. We need only recall that the order of gene transfer from this Hfr strain to a coli F⁻ mate is: T·L·Lac·Gal·λ. Under optimal conditions, mating can involve nearly 100 per cent of the Hfr cells. Zygotes that receive λ are lysed; when mating is allowed to go to completion, this lysis eliminates about 50 per cent of the zygotes. This system made it possible to compare zygotic induction and recombination using either Shigellas or *E. coli* as the F⁻. The same Hfr strain, but nonlysogenic, was used to

estimate recombination frequencies undisturbed by zygotic induction of λ (table 5). In the recombination experiments selection was made for Lac⁺Sʳ when using Shigellas F⁻ and for (TL)⁺Sʳ when using *E. coli* F⁻.

The results in table 5 show that, on the one hand, zygotic induction (and therefore mating and gene transfer) is about equally frequent in all cases. On the other hand, the recombination frequency is 100 to 1000 times lower in crosses with F⁻ Shigellas than with F⁻ *E. coli*. This is presumably not due to the different selective markers since, at least in crosses with the F⁻ *E. coli*, the Lac locus is donated to recombinants about 50 per cent as often as the TL segment (Wollman *et al.*, 1956). We conclude that the lower recombination reflects lower postzygotic integration of genes from the Hfr parent, rather than less frequent mating or less frequent transfer of genetic material. Test for a possible death of Shigella F⁻ cells following mating with *E. coli* Hfr gave negative results.

A remarkable observation is that the phage λ liberated upon zygotic induction in the mating of Hfr (λ) with *S. flexneri* strain 2aI is only partly (~50 per cent) modified to the λ·C form, although phage grown directly on strain 2aI is fully modified. In control experiments with *E. coli* strain C F⁻ the phage produced by zygotic

TABLE 5

Zygotic induction of phage λ and recombination frequencies

Organisms, Strain and Titers (cells/ml)	λ Plaques per ml of Mixtures				Recombination	
	40 Min		180 Min		Selection	Recombinants/ Hfr cells
	on C	on K	on C	on K		
Shigella dysenteriae Sh (1.2 × 10⁸) × *E. coli* Hfr (λ) (3.9 × 10⁷)	1.2 × 10⁷ *0.31*	7.7 × 10⁶	8 × 10⁸	4 × 10⁸	Lac⁺Sʳ	5.1 × 10⁻³
S. flexneri 2aI (2.6 × 10⁸) × *E. coli* Hfr (λ) (3.9 × 10⁷)	4.3 × 10⁶ *0.11*	2.6 × 10⁶	3.1 × 10⁸	1.4 × 10⁸	Lac⁺Sʳ	7.2 × 10⁻⁴
S. flexneri 2aI (λ) (1.7 × 10⁸) × *E. coli* Hfr (λ) (3.9 × 10⁷)	3.5 × 10⁶*	3.7 × 10⁵*	1.0 × 10⁸*	1.1 × 10⁷*	Lac⁺Sʳ	1.2 × 10⁻³
S. flexneri 2aI (λ) alone	3.1 × 10⁶	<10⁴	7.8 × 10⁷	<5 × 10⁵	—	—
E. coli F⁻(TL)⁻ (2.5 × 10⁸) × *E. coli* Hfr (λ) (3.9 × 10⁷)	5.6 × 10⁶ *0.14*	4.9 × 10⁶	6 × 10⁸	5 × 10⁸	(TL)⁺Sʳ	8.2 × 10⁻³
E. coli Hfr (λ) (3.9 × 10⁷) alone	≈7 × 10⁴	≈2 × 10⁵	≈4 × 10⁶	≈7 × 10⁶	—	—
E. coli F⁻(TL)⁻ (2.5 × 10⁸) × *E. coli* Hfr λˢ	—	—	—	—	(TL)⁺Sʳ	5.6 × 10⁻²

Zygotic induction. Growing cultures of SʳF⁻ bacteria were mixed with cells of *Escherichia coli* K-12 Hfr Sˢ (λ) (derived from Hfr H). The cells had been taken from growing cultures, chilled, spun and resuspended in broth. The mixtures were aerated. After 40 min of contact, samples were treated with anti-λ serum, diluted in broth and plated for λ plaques. A diluted sample was incubated for 180 min and plated again for λ plaques. All platings were done on λ-sensitive, Sʳ derivatives of both *E. coli* strain C and *E. coli* strain K-12, using agar with streptomycin.

Genetic recombination. Suitable dilutions of the same mixtures as above, or of mixtures with nonlysogenic Hfr, were plated on selective media after 40 min of contact in broth. When selection was for (TL)⁺Sʳ, the cells were washed and plated on minimal agar + thiamin + streptomycin.

Assays and controls. Similar cultures of unmixed parent strains were plated for viable counts after 30 min of incubation. They were also plated for λ plaques and on selective media as controls for zygotic induction and recombination, respectively.

The values in italics are the ratios "λ plaques/Hfr cells" where zygotic induction was present.

* Most plaques produced on C are accounted for by the lysogenic *S. flexneri* parent, and most of those on K by the lysogenic *E. coli* Hfr parent, without zygotic induction.

induction was more extensively modified to the λ·C form, although not as completely as reported by Jacob and Wollman (1956). Experiments are in progress to clarify the reason for the partial modification. It seems possible that a relation exists between the low level of postzygotic integration and the persistence of the λ·K form in coli × Shigella matings.

Note that zygotic induction occurs also in crosses with *S. dysenteriae* strain Sh, which cannot absorb phage λ and which, apparently, does not become λ-lysogenic upon mating. Growth of λ transduced by phage P1 into *S. dysenteriae* strain Sh has also been observed by Lennox (*to be published*). Zygotic induction of λ was absent, however, in a cross with *S. flexneri* strain 2aA F⁻.

Unstable hybrid strains. Most hybrid strains are stable for all selected and unselected parental markers. Moreover, each recombinant colony appears to be pure (nonsegregating) for all markers. The one exception concerns some crosses of *S. flexneri* strain 2aI and its derivatives with one strain of *E. coli* (Hfr $M^-V_6^r$ $V_{1,5}^r$). These crosses yield a certain proportion of unstable Lac⁺ colonies, which segregate out stable Lac⁻, unstable Lac⁺ and a few stable Lac⁺. The Lac⁺ unstable hybrids are stable for other characters derived from the Hfr parent (except sometimes for $V_{1,5}$, which is closely linked to Lac). It seems likely that in mutating from $V_{1,5}^s$ to $V_{1,5}^R$ the parent Hfr strain has acquired a factor resembling the Het factor described by Lederberg (1949). Further study of this factor is planned. The stable Lac⁺ segregants resemble other Lac⁺ recombinants from *S. flexneri* strain 2aI, except in a serological property to be discussed below.

Changes in mating polarity. Most F⁻ strains of *E. coli* can be converted to F⁺ by infection upon cultivation in admixture with F⁺ strain (Cavalli *et al.*, 1953; Hayes, 1953a; de Haan, 1954). This infection may be the expression of an incomplete mating (Wollman *et al.*, 1956). The conversion of Shigellas to the F⁺ state was accomplished and demonstrated by experiments of the type shown in table 6. Each of 3 Shigella strains was cultivated in mixture with an F⁺ strain of *E. coli* and reisolated at intervals by selective plating methods. Several of these isolates (and the original Shigella strains) were then subcultured with an F⁻ strain of *E. coli* (TLB₁)⁻. The coli strain was in turn reisolated and tested for fertility with *E. coli* F⁻M⁻. A positive test indicates

that the Shigella strain used had become F⁺ and a source of F⁺ contagion. The results were uniformly negative for the original Shigellas and positive for the treated ones, confirming the F⁻ nature of the former and their conversion to F⁺ by infection.

Repeated attempts, however, to demonstrate recombination between these F⁺ Shigellas and any F⁻ strain, either *E. coli* or Shigella, using a limited variety of selective methods, gave only negative results. Whether this is due to limitations of our tests or to an intrinsic property of F⁺ Shigellas cannot be decided from the available data.

Serological properties of hybrids. In agglutination tests for O-antigens, *E. coli* strain K-12 and its derivatives give only some slight cross reactions with the Shigella strains tested in our work.[2] When certain hybrids were tested with unadsorbed O-antisera against the parent strains, a new character appeared. Most Lac⁺ hybrids from crosses between *E. coli* strain K-12 derivatives and *S. flexneri* strain 2aI were found to give a reduced agglutinin titer with a commercial antiflexner "W" serum. This finding prompted further serological investigation of these and other hybrids.

Most of the Lac⁺ hybrids from crosses between *E. coli* (whether derivatives of strain K-12 or of strain C) and *S. flexneri* strain 2aI show the altered serological behavior with antiflexner sera, which consists in a reduced agglutinating titer with unadsorbed sera antiflexner W and antiflexner 2a, with increased agglutinability by several sera antiflexner 4a. These results are shown in table 7. Sera against other flexner prototypes were not tested.

Two Lac⁺ hybrids with the abnormal behavior, labeled strains F21 and F22, one λ-lysogenic, the other not, were selected and used to prepare antisera. These sera prove identical in range of reactivity. As shown in table 8 for the serum anti-F22, these sera gave high titers with the homologous hybrid strains and low titers with

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[2] Most cultures of *Escherichia coli* strain K-12 and its derivatives rapidly become rough under laboratory conditions. The sera were prepared with smooth cultures, which did not give self-agglutination in isotonic saline after overnight treatment with 70 per cent ethanol. In most agglutination tests the antigens were suspensions prepared from smooth colonies of an Hfr M⁻ derivative.

TABLE 6

Transfer of F$^+$ property between Escherichia coli and Shigella

Crosses (on Minimal Agar + Thiamin)	Recombinants
E. coli 　　F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)$^-$	—
E. coli$_I$ 　F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)$^-$	—
E. coli$_I$+ 　F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)$^-$	+
E. coli$_{II}$ 　F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)-	—
E. coli$_{II}$+ 　F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)$^-$	+
E. coli$_{III}$ 　F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)$^-$	—
E. coli$_{III}$+ F$^-$(TLB$_1$)$^-$ × *E. coli* F$^-$(MB)$^-$	+

Initial cultures: I = *S. dysenteriae* 57-1 S^r; II = *S. flexneri* 2aI S^r; III = *S. flexneri* 2aA S^r.

Mixed cultivation with *E. coli* F$^+$(TLB$_1$)$^-$S^s. Inoculum: 10^8 *E. coli* + 10^5 Shigella. Two or three daily transfers of 0.1 ml into 50 ml broth.

Reisolation of Shigellas I, II, III (F$^+$?) on streptomycin agar; labeled I$^+$, II$^+$, III$^+$.

Mixed cultivation of Shigellas I, II, III and I$^+$, II$^+$, III$^+$ with *E. coli* F$^-$(TLB$_1$)$^-$. Inoculum: 10^8 Shigella + 10^4 *E. coli*. Two daily transfers as above.

Reisolation of *E. coli* by washing and streaking on minimal agar plus thiamin. Labeled *E. coli*$_I$, *E. coli*$_I$+ . . . .

TABLE 7

Agglutination reactions of Shigella flexneri strain 2aI and of hybrids

Organism	Unadsorbed Sera*					
	Anti-W	Anti-2a	Anti-2b	Anti-4a		Anti-K-12
				Serum A	Serum B	
S. flexneri strain 2aI..................	25,600	25,600	1,600	400	1,600	<25
Hybrid F22.............................	400	6,400	1,600	3,200	6,400	<25
Escherichia coli strain K-12.............	<50	50	100	100	—	6,400

* Values are the highest serum dilutions with positive agglutination.

TABLE 8

Agglutination reactions of Shigella flexneri strains with various sera

Organism	Unadsorbed Sera					
	Anti-W	Anti-2a	Anti-2b	Anti-4a, Serum B	Antihybrid F22	Anti-K-12
S. flexneri 2aI.......................	25,600	25,600	1,600	1,600	400	<25
Hybrid F22...........................	400	6,400	1,600	6,400	6,400	<25
S. flexneri 2aA.......................	25,600	25,600	6,400	6,400	800	<25
Hybrids from 2aA...................	1,600	6,400	3,200	6,400	12,800	200
S. flexneri 2bA.......................	25,600	12,800	12,800	<200	400	<25
Hybrids from 2bA...................	1,600	6,400	3,200	12,800	12,800	50
S. flexneri 4aA.......................	1,600	6,400	3,200	12,800	12,800	400
Hybrids from 4aA...................	1,600	6,400	3,200	3,200	12,800	400
Escherichia coli K-12...............	<50	50	100	—	400	6,400

cultures of *S. flexneri* strains 2aI, 2aA and 2bA. Instead, the antihybrid serum agglutinates cultures of *S. flexneri* type 4a as much as its own homologous antigen. It agglutinates *E. coli* strain K-12 only with very low titer.

All Lac$^+$ hybrids from *S. flexneri* strain 2aI that were tested gave either the typical "F22-like" reactions shown in table 8 or normal 2a reactions, without intermediates. It was possible, therefore, to devise a simple test for extensive

screening of hybrids. This consists in inoculating each hybrid (from a pure restreaked colony) into 2 tubes with 1 ml of broth each, one containing serum anti-W diluted 1:3200, the other with serum anti-F22 1:800. Upon growth overnight at 37 C each hybrid is agglutinated in one or the other tube, but never in both. Several crosses between cultures of S. flexneri strain 2aI and derivatives of E. coli strain K-12 gave 95 per cent F22-like Lac+ hybrids and 5 per cent serologically unchanged Lac+ hybrids. Out of 100 hybrids selected for markers other than Lac+, the only two that were F22-like serologically were also Lac+.

Further crosses showed that most Lac+ hybrids between E. coli and another strain of S. flexneri type 2a (= 2aA) were serologically similar (although not identical) to the F22-like hybrids. That is, they had lost some titer with anti-2a and often also with anti-2b sera, and had generally become reactive at higher titer with serum anti-F22 and serum anti-4a. An unexpected finding was that very similar hybrid strains were obtained by crossing E. coli with S. flexneri type 2b. Also, hybrids with similar properties were obtained from crosses of E. coli with S. flexneri type 4a. The data are included in table 8.

These results suggested that all changed hybrids belonged to a single serological group. To assess the meaning of this hybrid group, we considered its serological relationship to E. coli strain K-12, to S. flexneri type 4a and to S. flexneri type Y. Tests on hybrids F21 and F22, kindly carried out by Dr. W. H. Ewing, classified these strains as S. flexneri type Y.

We tested all the hybrids, the parent cultures, and a culture of S. flexneri type Y with serum against E. coli K-12 and also with a number of anti-flexner sera that had been cross-adsorbed in various ways. The results, shown in tables 8 and 9, can be summarized as follows:

(1) The Y culture gives reactions similar to those given by the hybrids, although it is generally more reactive than any of them.

(2) E. coli strain K-12 cross-reacts to a limited but significant extent with S. flexneri type 4a; less with other type Shigellas. The hybrids vary somewhat in their reactions with anti-K-12 serum, but generally react with it less than cultures of flexner 4a.

(3) The agglutinins of antihybrid F22 serum cannot be adsorbed completely by the parent strains of S. flexneri type 2a nor by 2b, but are completely removed by adsorption with any one of several 4a strains and by a strain of S. flexneri Y.

(4) Adsorption of sera anti-2a or anti-2b with any one of the hybrids, as well as with the Y strain, removes heterologous reactions to the same extent.

(5) Adsorption of several anti-4a sera with any of the hybrids, including those derived from S. flexneri type 4a itself, as well as with Y, removes all agglutinins for 2a, 2b, Y and all the hybrids, leaving various amounts of homologous agglutinins, depending on the serum. Results with one serum are shown in tables 8 and 9. Other anti-4a sera are even more radically adsorbed by the Y and hybrid strains.

We conclude that the various hybrids, although probably not identical to one another, form a closely related group, similar to S. flexneri type Y, relatively closer to S. flexneri type 4a than to 2a or 2b, and whose antigenic structure includes the "group antigens" common to 4a, 2a and 2b. Note that such Y-like hybrid strains are obtained also from S. flexneri type 2b, which, according to its known antigenic composition, should, by loss of the type antigen, give rise to X-like variants, not to Y-like ones (Edwards and Ewing, 1955). This observation, and the fact that the agglutinins in anti-hybrid serum are only partially removed by adsorption with the parent S. flexneri type 2a (or 2b) prove that the serological change brought about by hybridization is not merely a loss of type antigen of the Shigella parent. The significance of these changes as to the possible origin and relations of various Shigella and E. coli serotypes will be discussed later.

The following points may be emphasized. First, the serological changes to the hybrid type are not due to the addition or substitution of a major E. coli antigen for the Shigella antigens in the hybrids, since cross reactions of the hybrids with E. coli strain K-12 are only slight. Apparently, the genetic factor(s) responsible for the change does not reach full phenotypic expression in E. coli, presumably because of the different genetic background. (Serological tests on E. coli strain C were prevented by its roughness.)

Second, the genetic factor(s) responsible for the change must be closely linked to the Lac locus, but are not located at the Lac locus, nor

TABLE 9

Agglutination reactions with unadsorbed and adsorbed flexner sera

Antigen	Serum*												
	2a	2a/H or 2a/Y	2a/4a	2b	2b/H or 2b/Y	2b/4a	4a, Serum B	4a/H or 4a/Y	4a/2aA	F22	F22/H or F22/Y	F22/4a	F22/2aI
Shigella flexneri strain 2aI†	25,600	6,400	6,400	1,600	1,600	1,600	1,600	<200	<200	400	<200	<200	<200
Hybrid F22	6,400	<200	400	1,600	<200	<200	6,400	<100	<200	6,400	<200	<200	1,600
Shigella flexneri strain 2aA†	25,600	12,800	6,400	6,400	3,200	800	6,400	<100	<200	800	<200	<200	<200
Hybrid from 2aA	6,400	<200	400	3,200	<200	<200	6,400	<100	400	12,800	<200	<200	3,200
Shigella flexneri strain 2bA†	12,800	6,400	3,200	12,800	12,800	6,400	<200	<100	<200	400	<200	<200	<200
Hybrid from 2bA	6,400	<200	400	3,200	200	200	12,800	<100	400	12,800	<200	<200	3,200
Shigella flexneri strain 4aA‡	6,400	<200	<200	3,200	<200	<200	12,800	>800	6,400	12,800	<200	<200	1,600
Hybrid from 4aA	6,400	<200	400	3,200	<200	<200	3,200	<100	200	12,800	<200	<200	3,200
Shigella flexneri Y	25,600	<200	400	12,800	<200	400	12,800	<100	400	12,800	<200	<200	6,400

* Adsorbed sera are indicated by the symbol/. For example, 2a/4a means: serum anti-2a adsorbed with 4a. /H means: serum adsorbed with any one of the four hybrids.

† The same results are obtained with the type cultures and with their S^r derivatives.

‡ The same results were obtained with two cultures received at several months interval from Dr. W. H. Ewing and with four single colony isolates from one of them.

are they another expression of the Lac⁺ property. In fact, about 5 per cent of the Lac⁺ recombinants are serologically unchanged. It is notable, although unexplained, that all stable Lac⁺ derivatives from the unstable Lac⁺ hybrids belong in the serologically unchanged group.

The linkage of the antigen-controlling factor to the Lac locus, used as a selective marker in our crosses, appears therefore to be a fortuitous coincidence. No other determinant of serological specificity has been detected yet in our rather limited range of crosses, using only a few strains of Shigella, a few selective markers, and rather crude serological tests. Further exploration is in order.

Attempted crosses Salmonella × E. coli. The existence of coliform, Lac⁺ cultures which ferment lactose in 24 hr, have antigens identical to those of *Salmonella newington* (group E₂, antigenic formula 3,15:e,h-1,6) and give rise to Lac⁻ variants (Seligmann and Saphra, 1946), suggested their possible origin as hybrids with *E. coli*. It seemed desirable to attempt crossing *E. coli* cultures F⁺ or Hfr with some Salmonellas belonging to group E₂. Cultures of *S. cambridge, S. newington, S. new brunswick, S. kinshase, S. selandia,* and of a Lac⁻ variant of culture #3534 (Saphra and Seligmann, 1947) were made Sʳ and plated on EMB lactose streptomycin agar with strains of *E. coli* Lac⁺ Sˢ, either F⁺ or Hfr. The results were uniformly negative.[3]

DISCUSSION

Many bacteria included in the genera *Escherichia* and *Shigella* have enough characteristics in common to suggest a close evolutionary relationship. They share, for example, susceptibility to certain phages and a common over-all pattern of catabolic reactions (although the fine details of catabolism and biosynthesis in Shigella remain largely unexplored). The main physiological differences, which are useful for practical purposes of classification because of stability and good correlation with presumed presence or absence of pathogenicity, include characters such as ability to utilize lactose. Yet, as stable as these characters

[3] Equally negative results were obtained in a attempt to obtain recombinants between *Escherichia coli* and a strain of *Pasteurella pestis,* kindly supplied by Dr. T. W. Burrows, who had observed that this strain and *E! coli* share susceptibility to several phages.

are in many strains, they are known to be controlled by genetic factors that are mutable in other strains. In the absence of hybridization tests, well established stable organisms can best be considered as "normotypes" for purposes of classification.

Our experiments have proved that Shigella can mate with *E. coli* and shares with it a common system of mating polarities. By hybridization we can create hybrids that would be considered as monstrosities from the standpoint of traditional bacterial classification, such as strains of *S. dysenteriae* that promptly ferment lactose (or arabinose or both), of *S. flexneri* that ferment lactose (and xylose or rhamnose) and so on. Once the already suspected existence of extensive genetic homology between coli and dysentery bacilli has been confirmed by hybridization, these findings are not at all surprising. Indeed, it should be possible, and might be desirable for practical reasons, to decide by genetic analysis the reason for the apparent stability of characters such as Lac⁻ in most strains of Shigella. Such stability may reflect intrinsic gene properties, or, more likely, the presence of multiple genetic blocks, or the absence of genetic loci (chromosomal deficiencies).

The failure of certain characters of *E. coli* parents to appear in hybrids suggests that the genetic homology is incomplete, and this may also be reflected in poor chromosomal pairing. Such poor pairing could underlie the low frequency of integration of *E. coli* genes into Shigella, in spite of the high frequency of mating revealed by zygotic induction. Similar observations have been reported in transformation experiments with Haemophilus; the frequency of integration of newly introduced genetic determinants is lower, the more distant the relationship between donor and recipient strains (Schaeffer, 1956). These observations have also been interpreted as reflecting inadequate pairing between genetic structures.

The availability of two methods of genetic recombination between *E. coli* and Shigella, mating and transduction by phage P1 (Lennox, 1955), makes it certain that the taxonomic structure of this group could be placed on a sound genetic basis. In the present state of bacterial genetics, however, when the nature of the mating process has just begun to be clarified (Wollman et al., 1956) and the genetic structure of only one or two strains of *E. coli* is known in some detail, a

detailed genetic analysis of any one Shigella would not seem too profitable. The establishment of a chromosomal basis for the evolution and taxonomy of this group of bacteria is a distant hope, although a definitely realizable one.

We now know that strains of Shigella and Escherichia are potentially interfertile organisms. Therefore, three main questions arise:

(1) How widespread is interfertility among other bacteria, at least among the *Enterobacteriaceae?*

(2) Does hybridization occur in nature, and if so, what role does the resulting gene flow play in the variability, survival, and evolution of these organisms?

(3) What role, if any, does hybridization play in creating the range of practically important normotypes of Shigella that occur in nature?

The answer to the first question, of the extent of potential interfertility among the bacterial groups, can only be guessed. Our few attempts to hybridize Salmonella and *E. coli* have failed, and no positive results seem to have been reported from elsewhere. The criterion of cross-sensitivity to phages, which probably reveals genetic homology in view of the relations between prophages and bacterial chromosomes (Stocker, 1955), is of limited use since few phages affect bacteria belonging to different "genera" of *Enterobacteriaceae* (except for Escherichia, Shigella, and related groups). The existence of unusual common properties (especially antigens) among strains classified in different groups may be a good guide to choice of cultures for further studies. These should take into account the possible existence of still unrecognized mating systems, with unsuspected polarities and restrictions in hybridizing capacity.

The question of the possible occurrence in nature of hybridization between Shigella and Escherichia, and possibly other groups, recalls suggestive observations by a number of workers. All experts in the diagnosis of enteric pathogens (Kauffmann, 1954; Weil and Saphra, 1953; Edwards and Ewing, 1955) have encountered cultures that appear to share properties of 2 (or more) well established normotypes and which could easily be explained by postulating a hybrid origin. To choose but a few examples, we may point first to entire groups of bacteria that fall into these "hybrid" classes: the Arizona group, which includes Suc$^+$, Lac$^+$ organisms that might

be recombinants between Salmonella and *E. coli*; the Alkalescens-Dispar group, nonaerogenic, nonmotile organisms, including Lac$^+$ ones, which might be hybrids between *E. coli* and *S. flexneri*. Also some cultures (Seligmann and Saphra, 1946; Saphra and Seligmann, 1947) ferment lactose as promptly as *E. coli* but are antigenically identical to known Salmonella strains and can, by a single mutation Lac$^+$ → Lac$^-$, become indistinguishable from the latter organisms. Likewise, organisms of the Alkalescens-Dispar group are antigenically related to certain *S. flexneri* normotypes on the one hand, to typical *E. coli* strains on the other hand.

Individual strains with O-antigens in common with known coli, flexneri and Salmonella cultures have been described (Bernstein *et al.*, 1941; Saphra and Wassermann, 1945). Coliform organisms related to Shigellas (Ewing, 1953) have raised important questions concerning pathogenicity and diagnosis (Stuart *et al.*, 1943). The experts have been unanimous in emphasizing the existence and importance of "intermediate" organisms inbetween the major normotypes (Edwards and Ewing, 1955). So long as vegetative reproduction was thought to be the only reproductive process in bacteria, most authors concerned with bacterial evolution have interpreted these intermediate organisms as variants fulfilling or recapitulating a mutational history of the *Enterobacteriaceae*. Yet, the possibility of a hybrid origin has not escaped some workers. For example, Saphra and Wassermann (1945) state: ". . . originally highly different forms might have a tendency of acquiring more and more similarity of antigenic properties. One might designate such a working hypothesis as a 'hypothesis of convergent development' in contrast to the 'hypothesis of divergent development' of White. Influences connected with the adjustment of life in the intestinal tract and possibly the influence of one species upon another during their co-existence in the intestinal tract might favor such a development. The possibility that one species might impress its antigen upon another has been experimentally proven in the classical transformation experiments on pneumococci by Griffith and by Dawson."

Indeed, the normal habitat of *Enterobacteriaceae*, that is, the intestine of mammals and other animals, is quite conducive to hybridization, because of the presence of ubiquitous *E. coli*

strains in populations of enormous size and of the chances for numerous collisions (and other interactions, such as phage transfer) among cells of these strains and of any newcomer organism. Our experiments establish the reality of the genetic interaction and justify the search for its occurrence in nature and for the role that the resulting gene flow may play in bacterial evolution. This task has more than purely biological or genetic interest. It may hold the key to the whole epidemiology of enteric diseases; it adds many potential dimensions to the tremendously important work of tracing and identifying the enteric pathogens.

Thus, for example, all our Lac⁺ hybrids, although they are predominantly "Shigellas" (and probably still pathogenic) would never be detected as potential pathogens by the routine diagnostic procedures. They would be discarded as "coliforms" and might not even be tested for anaerogenesis, (which might be considered as evidence of a possible hybrid nature) except if isolated from cases of infantile diarrhea. Indeed, several of the "coliforms" supposedly responsible for diarrhea are related antigenically to some Shigellas (Edwards and Ewing, 1955) and may be hybrid strains. Also, for example, a coliform organism antigenically related to S. flexneri type 2 was isolated from the stool of a patient who also yielded a typical, stable S. flexneri type 2 (Ewing, 1953, and personal communication). A systematic survey for hybridizable organisms and hybridization phenomena in nature, carried out with controlled genetic methods, would be readily feasible and should be fertile of remarkable findings and applications.

A question closely related to the above discussion concerns the possible hybrid origin of some of the well known Shigella normotypes. Our serological findings establish that hybridization of S. flexneri type 2a or 2b with E. coli can bring about at least one major antigenic change, which is controlled by a genetic determinant near the Lac region of the coli-Shigella chromosome, and which produces a group of serotypes, the F22-like hybrids, similar to S. flexneri type Y. The association of the new serotype with the Lac⁺ property is clearly a fortuitous accident of genetic linkage. Lac⁻ hybrids with F22-like serotype would not have been detected in our crosses. Likewise, if Lac⁺ hybrids of such serotype occurred in nature, they would probably be identified as Shigellas only if they happened to

mutate to Lac⁻. In view of the finding of one such major change in our limited range of experiments, it would be surprising if antigenic changes of some sort were not quite frequent following hybridization. Only a systematic study of the distribution of mating abilities among Shigella and E. coli strains in nature can help evaluate this possibility.

The similarity of the hybrid serotype to S. flexneri type Y raises interesting questions concerning the much debated significance of the Y organisms. According to Boyd (1938) and others (Edwards and Ewing, 1955) the Y strains should be considered as variants derived from various S. flexneri types by loss of the type antigens. Such variation has been observed in the laboratory, for example, in S. flexneri types 4a (Boyd, 1938) and 2a (Ewing, 1954). Other authors (Weil and Saphra, 1953) consider these variants as different from S. flexneri type Y, which they report as possessing a type antigen of its own.

Our Y-like hybrids, derived from either 2a, 2b, or 4a type strains, cannot be simply loss variants, because they have antigenic relations qualitatively different from those of the parent cultures; this is especially evident for the hybrid derived from S. flexneri type 2b. The genetic aspects of the phenomenon, namely its dependence on a genetic determinant linked with a specific region of the genome, indicate that the serological change reflects a new combination of antigen-controlling genes, probably due to the introduction of coli genetic elements into Shigella. The relation of this variation by hybridization to similar spontaneous variations occurring in pure lines of S. flexneri remains unclarified. It is possible that both types of genetic changes reveal hidden antigenic potentialities of the strains of origin.

There seems to be no definite evidence to show that the O-antigens of Shigellas consists of a mosaic of different antigenic determinants. The various antigens are defined only in terms of serological agglutinations and cross-adsorption tests. If the O-antigen (the carbohydrate-lipoprotein complex) of each organism owed its antigenic specificity to a single molecular species (Morgan and Partridge, 1940), then the various antigenic components, as defined by cross-reactions, would be the expression of the differential affinities between the unique antigen of each organism, probably determined by several

genes, and the various fractions of a population of antibody molecules that includes a whole spectrum of configurations (Landsteiner, 1945). Each strain would react with, and could adsorb from, a serum those antibody molecules that fit it. The greater the proportion of such molecules in a serum, the stronger will be the cross-reaction and the closer the inferred chemical similarity between the antigens of the homologous and of the heterologous strain. Mutation or recombination of genetic determinants could give rise to new antigens with new specificities.

If, on the other hand, the O-antigens were a mosaic of different reactive sites, then mutation or recombination could act by changing one or more of these sites, while leaving the other unaffected. Such a mosaic structure cannot be proved by genetic tests, but only by the demonstration of the existence of separable combining sites for different antibody molecules in the O-antigen of a given strain, either on the intact cell or in the extracted O-antigen complex.

SUMMARY

A number of Shigella strains were tested for mating ability with *Escherichia coli*. All the strains tested were fertile with F^+ or Hfr derivatives of *E. coli* strain K-12 and *E. coli* strain C. The Shigellas behave like F^- strains in the fertility system of *E. coli* and, like *E. coli* F^-, can be changed to the F^+ state by mixed cultivation with an F^+ culture. Matings result in formation of hybrids, which exhibit new combinations of characters typical of Shigellas with characters typical of *E. coli*. The frequency of recombination is lower than in similar crosses between strains of *E. coli*, although the frequency of mating, measured by zygotic induction of prophage λ, is comparable in both types of crosses. Also, some of the characters of the coli parent fail to be transmitted to the *E. coli* × Shigella hybrids. These results suggest an incomplete genetic homology between the two groups of organisms.

Some hybrids between *E. coli* and type strains of *Shigella flexneri* possess somatic antigens closely related to those of *S. flexneri* type Y. This antigenic change is controlled by genetic factors closely linked to factors controlling lactose utilization. The results suggest a possible role of hybridization occurring in nature in the evolution of the *Enterobacteriaceae* and in the origin of aberrant and intermediate strains of enteric bacteria.

REFERENCES

BERTANI, G. 1951 Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol., **62**, 293–300.

BERTANI, G. AND WEIGLE, J. 1953 Host-controlled variation in bacterial viruses. J. Bacteriol., **65**, 113–121.

BERNSTEIN, S., SAPHRA, I., AND DANIELS, J. B. 1941 The occurrence of Salmonella antigens in dysentery bacilli. J. Immunol., **42**, 401–404.

BOYD, J. S. K. 1938 The antigenic structure of the mannitol-fermenting group of dysentery bacilli. J. Hyg., **38**, 477–499.

CAVALLI, L. L., LEDERBERG, J., AND LEDERBERG, E. M. 1953 An infective factor controlling sex compatibility in *Bacterium coli*. J. Gen. Microbiol., **8**, 89–103.

CAVALLI-SFORZA, L. L. AND JINKS, J. L. 1956 Studies on the genetic system of *Escherichia coli* K-12. J. Genetics, **54**, 87–112.

COHN, M. 1952 Production of antibodies in experimental animals. In *Methods in medical research*, Vol. 5. Year Book Publishers, Chicago, Ill.

EDWARDS, P. R. AND EWING, W. H. 1955 *Identification of Enterobacteriaceae*. Burgess Publishing Co., Minneapolis, Minn.

EWING, W. H. 1953 Serological relationships between Shigella and coliform cultures. J. Bacteriol., **66**, 333–340.

EWING, W. H. 1954 Mannitol negative varieties of *Shigella flexneri* serotypes. J. Immunol., **72**, 404–410.

DE HAAN, P. G. 1954 Genetic recombination in *Escherichia coli* B. I. The transfer of the F agent to *E. coli* B. Genetica, **27**, 293–299.

HAYES, W. 1953a Observations on a transmissible agent determining sexual differentiation in *Bacterium coli*. J. Gen. Microbiol., **8**, 72–88.

HAYES, W. 1953b The mechanism of genetic recombination in *Escherichia coli*. Cold Spring Harbor Symposia Quant. Biol., **18**, 75–93.

JACOB, F. AND WOLLMAN, E. L. 1956 Sur les processus de conjugaison et de recombinaison chez *Escherichia coli*. I. L'induction par conjugaison ou induction zygotique. Ann. inst. Pasteur, **91**, 486–510.

KAISER, A. D. 1955 A genetic study of the temperate coliphage λ. Virology, **1**, 424–443.

KAUFFMANN, P. 1954 *Enterobacteriaceae*. 2nd ed. Ejnar Munksgaard, Copenhagen.

LANDSTEINER, K. 1945 *The specificity of serological reactions.* Harvard Univ. Press, Cambridge, Mass.

LEDERBERG, J. 1947 Gene recombination and linked segregations in *Escherichia coli.* Genetics, **32**, 505–525.

LEDERBERG, J. 1949 Aberrant heterozygotes in *Escherichia coli.* Proc. Natl. Acad. Sci. U. S., **35**, 178–184.

LEDERBERG, J. 1950a Isolation and characterization of biochemical mutants of bacteria. In *Methods in medical research*, Vol. 3. Year Book Publishers, Chicago, Ill.

LEDERBERG, J. 1950b The β-D-galactosidase of *Escherichia coli* strain K-12. J. Bacteriol., **60**, 381–392.

LEDERBERG, J. 1951 Prevalence of *Escherichia coli* strains exhibiting genetic recombination. Science, **114**, 68–69.

LEDERBERG, J. AND EDWARDS, P. R. 1953 Serotypic recombination in Salmonella. J. Immunol., **71**, 232–240.

LEDERBERG, J. AND TATUM, E. L. 1946 Novel genotypes in mixed cultures of biochemical mutants of bacteria. Cold Spring Harbor Symposia Quant. Biol., **11**, 113–114.

LEDERBERG, J., LEDERBERG, E. M., ZINDER, N. D., AND LIVELY, E. R. 1951 Recombination analysis of bacterial heredity. Cold Spring Harbor Symposia Quant. Biol., **16**, 413–443.

LENNOX, E. S. 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology, **1**, 190–206.

LIEB, M., WEIGLE, J. J., AND KELLENBERGER, E. 1955 A study of hybrids between two strains of *Escherichia coli.* J. Bacteriol., **69**, 468–471.

LURIA, S. E. 1947 Recent advances in bacterial genetics. Bacteriol. Revs., **11**, 1–40.

MORGAN, W. T. J. AND PARTRIDGE, S. M. 1940 Studies in immunochemistry. 4. The fractionation and nature of antigenic material isolated from *Bacterium dysenteriae* (Shiga). Biochem. J. (London), **34**, 169–191.

SAPHRA, I. AND SELIGMANN, E. 1947 Coliforms with complete Salmonella antigens, or lactose-fermenting Salmonellas. J. Bacteriol., **54**, 270–271.

SAPHRA, I. AND WASSERMANN, M. 1945 Serological relationship amongst Salmonella and other Enterobacteriaceae. J. Immunol., **50**, 221–227.

SCHAEFFER, P. 1956 Transformation interspécifique chez des bactéries du genre Hemophilus. Ann. inst. Pasteur, **91**, 193–211.

SCHAEFFER, P. AND RITZ, M. E. 1955 Transfer interspécifique d'un charactère héréditaire chez des bactéries du genre Hemophilus. Compt. rend. Acad. Sci. **240**, 1491–1493.

SELIGMANN, E. AND SAPHRA, I. 1946 A coliform bacterium with the complete antigens of *Salmonella newington.* J. Immunol., **54**, 275–282.

STOCKER, B. A. D. 1955 Bacteriophage and bacterial classification. J. Gen. Microbiol., **12**, 375–381.

STUART, C. A., RUSTIGIAN, R., ZIMMERMAN, A., AND CORRIGAN, E. V. 1943 Pathogenicity, antigenic relationships and evolutionary trends of *Shigella alkalescens.* J. Immunol., **47**, 425–437.

WEIL, A. J. AND SAPHRA, I. 1953 *Salmonellae and Shigellae.* Charles C Thomas, Springfield, Ill.

WOLLMAN, E. L., JACOB, F., AND HAYES, W. 1956 Conjugation and genetic recombination in *Escherichia coli* K-12. Cold Spring Harbor Symposia Quant. Biol., **21**, 141–162.